stratec
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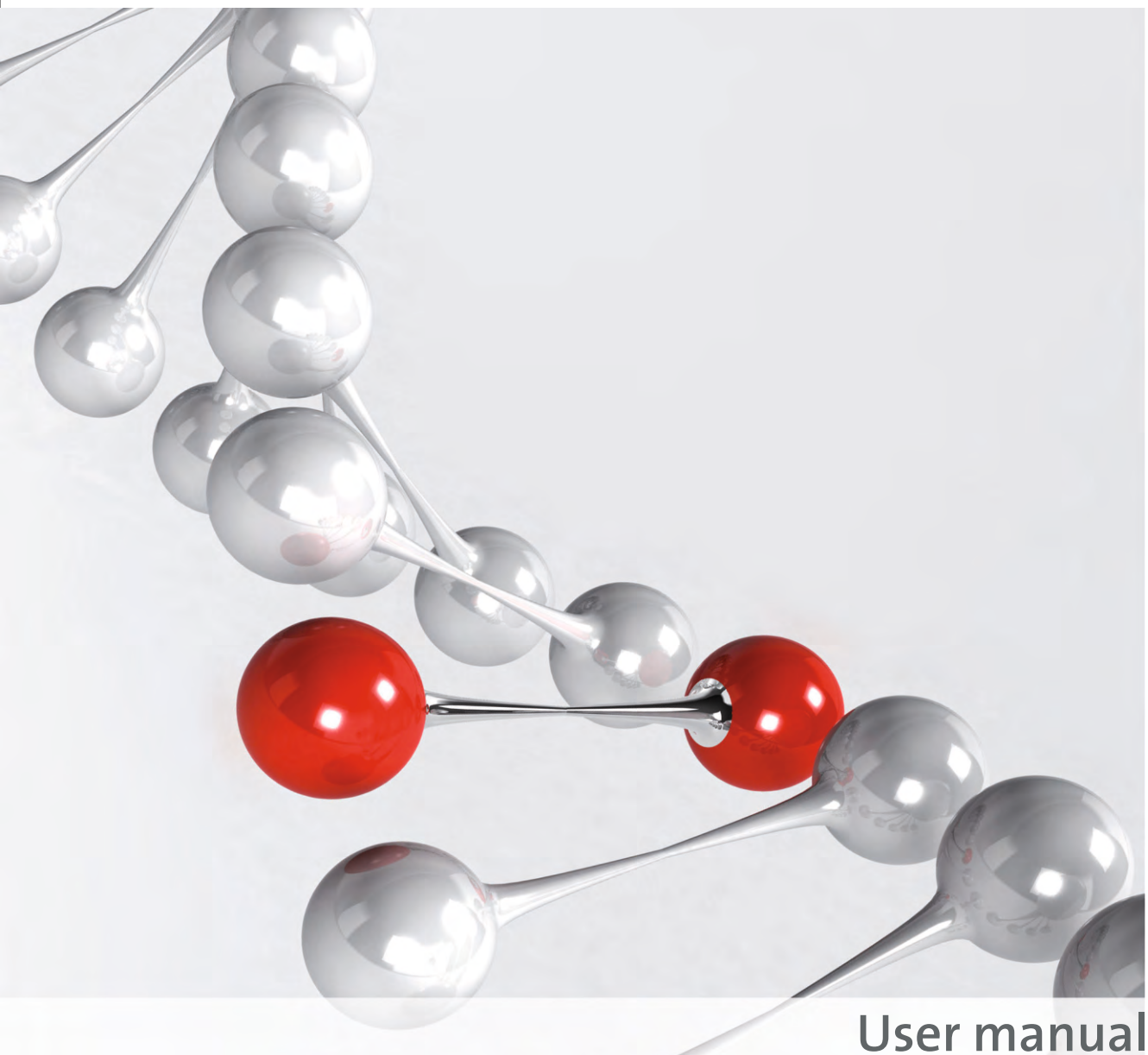


User manual
InviMag® Virus RNA Mini Kit/ IG
for use on the InviGenius®, STRATEC Molecular GmbH

for automated purification of viral RNA from serum, plasma, cell-free body fluids, rinse liquid from swabs & stool samples with magnetic beads

CE
IVD

REF 2443120100

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviMag® Virus RNA Mini Kit/ IG

The **InviMag® Virus RNA Mini Kit/ IG** combines the advantages of the innovative Invisorb® technology with easy handling of magnetic particles for a very efficient and reliable isolation of viral RNA with high purity in a fully automated process.

The nucleic-acid-binding magnetic particles are characterized by a high specific surface area, a uniform size distribution and good suspension stability. Therefore, the particles are highly suitable for high throughput processing of nucleic acids.

The **InviMag® Virus RNA Mini Kit/ IG** is the ideal tool for fully walk-away isolation and purification of viral RNA from 12 samples of fresh or frozen plasma, serum, cell-free body fluids as well as rinsed liquid from swabs and supernatant from stool suspension with the InviGenius® fully automated extraction system.

The interplay of the RNA extraction and purification chemistry, provided by the **InviMag® Virus RNA Mini Kit/ IG** with the InviGenius® system, was intensely tested.

CE **IVD** Compliance with EU Directive 98/79/EC on *in-vitro* medical devices.

Not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in-vitro medical devices is not recognized.

Contents

Kit contents of InviMag® Virus RNA Mini Kit /IG

Store the **MAP Solution B** at 4°C!
Store lyophilized **Proteinase K/ Carrier-RNA (PKC Tube)** at 2 - 8 °C!
Store dissolved **Proteinase K/ Carrier-RNA** at –20 °C!
Store all other kit components at room temperature (RT)!

	8 x 12 extractions	**reagents sufficient for**
Catalogue Number	2443120100	
Lysis Buffer RV	60 ml	8 runs per bottle
PKC-Tube	for 8 x 800 µl working solution	1 vial per run
RNAse Free Water	15 ml	
MAP Solution B/ IG	2 x 2.6 ml	1 vial per run
Binding Solution (fill with 99.7% Isopropanol)	empty bottle (final volume 60 ml)	8 runs
Wash Buffer R1	60 ml (final volume 120 ml)	8 runs
Wash Buffer R2	2 x 25 ml (final volume 2 x 125 ml)	4 runs
Elution Buffer R	30 ml	8 runs
Sealing Oil	25 ml	8 runs
Incubation Plate A	1	8 runs
Working Plate A	4	2 runs
Elution Plate E	1	8 runs
Microtube Cap	8	
Sheath Box	1 (2 racks á 48 sheaths)	4 runs per plate (48 pieces)
Sealing Foils	4	
Incubator Stripe Foils	2	
Initial steps	Add 60 ml of 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle labelled “Binding Solution” Add 60 ml of 96-100% ethanol to the bottle **Wash Buffer R1**. Add 100 ml of 96-100% ethanol to each bottle **Wash Buffer R2**. Mix thoroughly and always keep the bottles firmly closed! Add 800 µl of the provided RNase free Water to each **PKC** vial and mix. Only prepare as much vials as required for a run.	

Symbols

Lot number

Catalogue number


Expiry date


Consult operating instructions


Temperature limitation


Do not reuse

Storage

All buffers and kit contents of the **InviMag® Virus RNA Mini Kit / IG,** except **PKC Tube** and **MAP Solution B** should be stored at room temperature and are stable for at least 12 months.

PKC Tube: Lyophilized **PKC Tube** should be stored at 2-8°C. If dissolved in RNase-free water storage at -20°C is recommended.

MAP B Solution: The magnetic beads should be stored at 4°C.

Wash Buffers: Wash Buffers charged with ethanol should be stored at room temperature and should be appropriately sealed. If any precipitates are visible within the provided solutions, solve them by carefully warming up to 30°C.

Room temperature (RT) is defined as range from 15-30°C.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviMag® Virus RNA Mini Kit/ IG** for applications as described in this manual. Purchaser must determine the suitability of the product for its particular use. Should any product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, the product will be replaced free of charge.

STRATEC Molecular reserves the right to change, alter, or modify any product to enhance its performance and design at any time.

In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviMag® Virus RNA Mini Kit/ IG** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.

If you have any questions or problems regarding any aspects of **InviMag® Virus RNA Mini Kit/ IG** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:
from Germany: +49-(0)30-9489-2901/ 2910
from abroad: +49-(0)30-9489-2907
or contact your local distributor.

Intended use

The **InviMag® Virus RNA Mini Kit/ IG** is designed for fully automated extraction and purification of viral RNA from up to 200 µl serum or plasma based samples. Up to 12 samples can be processed using a patented magnetic beads system and the InviGenius® robotic platform.

It is advised to provide at least 550 µl sample per tube (dead volume) to prevent pipetting distribution errors due to the liquid level detection (LLD) process. The final processed sample volume is 200 µl. The nucleic acid isolation protocol is suitable for routinely walk-away automated preparation of viral DNA from fresh or frozen sample. For reproducible and high yields an appropriate sample storage is essential (see “Sampling and storage of the starting material”, page 9).

Common collection tubes (not provided) and anticoagulants (EDTA and citrate, *but not heparin*) can be used to assemble a set of samples. All utilities (reagents and plastics) – except conductive tips - required for preparation of viral RNA are provided by the **InviMag® Virus RNA Mini Kit/ IG**.

THE PRODUCT IS ITDENDED FOR USE BY PROFESSIONALS, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in-vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in-vitro medical devices is not recognized.

Product use limitation

The kit is validated for viral RNA extraction from cell-free body fluids and rinsed liquids, specifically for human serum and plasma. Related applications will need a separate validation. Extraction of eukaryotic RNA from samples has not been evaluated with this kit.
The included chemicals are only useable once.
Differing of starting material may lead to inoperability. Therefore, neither a warranty nor guarantee in this case will be given, implied or expressed.
The user is responsible to validate the performance of the STRATEC Molecular product for any particular use. STRATEC Molecular does not provide validations of performance characteristics of the product with respect to specific applications. STRATEC Molecular products may be used e.g.in clinical diagnostic laboratory systems conditioned upon the complete diagnostic system of the laboratory the laboratory has been validated pursuant to CLIA’ 88 regulations in the U.S. or equivalents in other countries.
All products sold by STRATEC Molecular are subject to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485) and are warranted to perform as described herein. Any problems, incidents or defects shall be reported to STRATEC Molecular immediately upon detection thereof.
The chemicals and the plastics are for laboratory use only. They must be stored in the laboratory and must not be used for other purposes than intended.
The product with its contents is not suitable for consumption.

Safety information

When and while working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles!

Avoid skin contact! Adhere to the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular product and its components. If buffer bottles are damaged or leaking, **WEAR GLOVES, AND PROTECTIVE GOGGLES** when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the waste generated by the **InviMag® Virus RNA Mini Kit/ IG** procedures for residual infectious materials. Contamination of the waste with residual infectious materials is highly unlikely, but cannot be excluded completely. Therefore, all waste has to be considered infectious and should be handled and discarded accordingly to local safety regulations.

European Community risk and safety phrases for the components of the **InviMag® Virus RNA Mini Kit/ IG** to which they apply, are listed below as follows:

Lysis Buffer RV

warning
H302-312-332-412 EUH032 P273

Proteinase K:

danger
H315-319-334-335 P280-305-351-338-310-405

Wash Buffer R1

danger
H302-312-332-412 EUH032 P273

H302:	Harmful if swallowed.
H312:	Harmful in contact with skin.
H332:	Harmful if inhaled.
H412:	Harmful to aquatic life with long lasting effects.
H315:	Causes skin irritation.
H319:	Causes serious eye irritation.
H334:	May cause allergy or asthma symptoms or breathing difficulties if inhaled.
H335:	May cause respiratory irritation.
EUH032:	Contact with acids liberates very toxic gas.
P273:	Avoid release to the environment.
P280:	Wear protective gloves/protective clothing/eye protection/face protection.
P305+P351+P338:	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if Present and continue rinsing.
P310:	Immediately call a POISON CENTER or doctor/physician.
P405:	Store locked up.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: 1 – 352 – 323 – 3500
inside of USA : 1 – 800 – 535 – 5053

Product characteristics of the InviMag® Virus RNA Mini Kit/ IG

The **InviMag® Virus RNA Mini Kit/ IG** is an ideal tool for efficient and fully automated viral RNA extraction and purification from fresh or frozen samples using magnetic beads in combination with the InviGenius® system.

Starting material	Yield	Time for preparation
200 µl serum or plasma; 200 µl cell free body fluids; 200 µl rinse liquid from swab; 200 µl supernatant from cell cultures 50 mg stool sample (supernatant from stool suspension)	depends on the sample (source and storage) ***Note:*** *The added Carrier-RNA (PKC tube) will account for most of the eluted RNA. Quantitative RT-PCR is recommended for determination of the viral RNA yield.*	~ 70 min for 12 samples

The RNA isolation process is based on the interaction of nucleic acids with silica coated magnetic particles at adapted buffer conditions. The InviGenius® instrument will automatically perform all steps of sample and reagent distribution. The RNA purification procedure is performed without any user intervention, except the initial loading of the system, thus allowing safe handling of potentially infectious samples. Sample cross-contamination and reagent cross-over is effectively eliminated by the automated purification process. The use of unique bar codes for samples and reagents avoids unwanted transpositions.

The InviGenius® instrument uses magnetic rods to transport the RNA-binding magnetic particles through the various extraction phases: lysis, binding, washing and elution. The volume of buffers and other liquids necessary for RNA isolation is reduced to a minimum. Eliminating the direct liquid handling and increasing the automation level results in a fast, reliable and robust technique.

After a sample specific lysis - using **Lysis Buffer RV** and **PKC** - optimal binding conditions are adjusted by addition of **Binding Solution.** The viral RNA binds to the simultaneously added magnetic particles and is separated from the solution by the magnetic rods controlled by the InviGenius® system. Subsequent to three washing steps of the particle bound nucleic acids using **Wash Buffer R1** and **Wash Buffer R2**, the DNA is finally eluted in **Elution Buffer R**.

Due to the high purity, the eluted viral RNA is ready-to-use in a broad panel of downstream applications such as:

- real-time PCR* (quantitative RT-PCR, like TaqMan® und LightCycler® technologies)
- or array technologies

For the isolation of DNA from a single serum, plasma or blood sample STRATEC Molecular offers the **Invisorb® Spin Virus RNA Mini Kit** or for 8–96 samples the **Invisorb® Virus RNA HTS 96 Kits** for use on a centrifuge, vacuum manifold or other robotic workstations.

For further information please contact: phone +49 (0) 30 9489 2901, 2910 in Germany and from foreign countries phone +49 (0) 30 9489 2903, 2907 or ask your local distributor.

Sampling and storage of starting material

Best results are obtained using freshly extracted samples. As long as the samples are not shock-frosted with liquid nitrogen or incubated with RNase inhibitors or denaturing reagents, the viral RNA is not secured. It is essential, that samples are processed as fast as possible on the InviGenius. Long term storage at –80°C is recommended.

Serum and plasma
After collection and centrifugation, serum and plasma from blood (treated with anticoagulants like EDTA or citrate, but **not** with heparin), synovial fluid samples or other cell-free body fluids, swabs as well as stool samples can be stored on ice for 1-2 hours. For a short time (up to 24 h) samples may be stored at -20°C. For long-term storage, we recommend freezing samples in aliquots at –80°C. Frozen plasma or serum samples must not be thawed more than once. Multiple thawing and freezing before isolating the viral RNA should be avoided. It may lead to denaturation and precipitation of proteins, resulting in reduced viral titers and therefore reduced yields of viral Nucleic acids. In addition, cryoprecipitates formed during freeze-thawing can give problems. If cryoprecipitates are visible, they should be centrifuged at app. 6.800 x g for 3 minutes. The cleared supernatant should be aspirated, without disturbing the pellet and be processed immediately. This step will not reduce viral titers.

Stool
Best results are obtained with fresh material. The collected fresh stool sample can be stored at ambient temperature for at least 1-2 hours at RT, but the high content of DNases and RNases can lead to a quick digestion and degradation of the viral RNA. The sample should be quickly prepared and processed on the InviGenius®. Alternatively, it can be stored frozen at – 80°C for weeks or months.

Swabs
The protocol works with fresh prepared swabs as well as with dried swabs. The protocol has not been validated for isolation of RNA from swabs which are stored in special storage buffers of other providers.

STRATEC Molecular will not take responsibility if other sample types than described above are used or if the sample preparation advices are modified

Principle and procedure

The **InviMag® Virus RNA Mini Kit/ IG** procedure comprises following steps after loading of the samples and buffers and starting the automated process:

- Lysis of the virus particles
- Binding of the viral RNA to the magnetic beads
- Washing and evaporation of ethanol
- Elution of viral RNA

After lysis, the viral RNA binds to the magnetic beads whereas contaminations and enzyme inhibitors are efficiently removed during the following three wash steps and highly purified viral RNA is eluted finally.

This manual contains 3 protocols.

Procedure

Lysis

Samples are lysed at denaturing conditions in an Incubation Plate A at elevated temperatures in the presence of **Lysis Buffer RV** and a **PKC** mixture.

Binding of the viral nucleic acids

After adding **Binding Solution** and **MAP Solution B** to the lysate, the viral RNA is bound to the surface of the magnetic beads.

Removing residual contaminants

Contaminants are efficiently removed using **Wash Buffer R1** and **R2**, respectively, while the nucleic acids remain bound to the magnetic beads.

Elution

The viral RNA is finally eluted from the beads using 100 µl **Elution Buffer R** and transferred to the **Elution Plate E**. The bottom-magnets guarantees a magnetic-beads-free eluate which is ready-to-use in different downstream applications like real-time PCR** (quantitative RT-PCR, like TaqMan® und LightCycler® technologies) or array technologies.

Yield and quality of viral RNA

The amount of purified RNA in the **InviMag® Virus RNA Mini Kit/ IG** procedure depends on the sample type, the virus content, sample source, transport, storage, and age.

Yield and quality of isolated viral RNA is suitable for any molecular diagnostic detection system. The diagnostic tests should be performed accordingly to the manufacturers' specifications.

Note: *If beads are visible in the eluate, transfer the eluate to a new reaction tube and centrifuge for 1 min at maximum speed (e.g.13000 rpm).*

Different amplification systems vary in efficiency depending on the total amount of nucleic acid present in the reaction. Eluates from this kit contain both viral RNA and Carrier-RNA whereas the amount of Carrier-RNA will greatly exceed the amount of viral nucleic acids.

Yields of viral RNA isolated from biological samples are normally less concentrated than 1 µg and therefore impossible to determine photometrically*. Keep in mind that the added Carrier-RNA (~ 5 µg per 200 µl sample) will account for most of the present RNA.

The kit is suitable for downstream analysis with NAT techniques, for examples qPCR, RT qPCR, LAMP, LCR. Diagnostic assays should be performed accordingly to the manufacturer's instructions.

Quantitative RT-PCR is recommended for determination of viral RNA yield.

*) In Gel Electrophoresis and in Capillary Electrophoresis, RNA extracted with the provided kit looks like degraded cause the kit contains Carrier RNA, this is poly A RNA in fragments of 100 up to 1000 bases. The kit is not dedicated for applications using this kind of analysis.

**) The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

Important notes

Important points before starting a protocol

Important points before starting a protocol

Immediately upon arrival of the product, inspect the kit and its components as well as the package for any apparent visible damages and correct quantities. If there are any unconformities, please notify STRATEC Molecular in writing with immediate effect upon inspection thereof. If buffer bottles are damaged, contact the STRATEC Molecular Technical Services or your local distributor. In case of liquid spillage, refer to “Safety Information” (see page 6). Do not use damaged kit components, since their use may lead to poor kit performance.

- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Discard contaminated gloves immediately
- Do not combine components of different kits.
- Avoid microbial contaminations of the kit reagents.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow.
- This kit should only be used by trained personnel.

Preparing reagents and buffers

Before starting a run, bring all reagents to room temperature. Where necessary, gently mix and redissolve any precipitates by incubating at 30°C. Swirl gently to avoid foaming.

Lysis Buffer RV, MAP Solution B and **Elution Buffer R** are ready-to-use.

8 x 12 viral RNA extractions
Add 60 ml of 99.7% **Isopropanol** (molecular biologic grade) into the empty bottle
Resuspend lyophilized **PKC** by addition of 800 µl of the provided RNase/DNase free water, mix thoroughly and store diluted and unused **PKC** at -20°C.Avoid repeated freezing and thawing cycles which will degrade the Carrier-RNA and reduce the functionality of the Kit.
Add 60 ml of 96-100% ethanol to the bottle **Wash Buffer R1**. Mix thoroughly and always keep the unused bottle firmly closed!
Add 100 ml of 96-100% ethanol to each bottle **Wash Buffer R2**. Mix thoroughly and always keep unused bottles firmly closed!

Internal Extraction Control: please check the instruction on page 14.

Reagents and equipment to be supplied by user

- Measuring cylinder (250 ml)
- Pipette tips
- Disposable gloves
- PBS buffer
- ddH_2O
- Vortexer
- 96-100% ethanol
- Isopropanol*

*) The **InviMag® Virus RNA Mini Kit/ IG** is validated with 2-Propanol; Rotipuran >99.7%, p.a., ACS, ISO (Order no. 6752) from **Carl Roth.**

Possible suppliers for Isopropanol

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

Possible primary tubes, manufacturer, cat. no.

Venosafe, 5.5 ml, Ref, VF-076SDK, Terumo
Vacuette, 2 ml, Ref, A110500I, Greiner bio-one
Vacuette, 9 ml, Ref, 455036, Greiner bio-one
BD Vacutainer, 2.7 ml, Ref, 363048
BD Vacutainer, 6 ml, Ref, 367864
BD Vacutainer, 10 ml, Ref, 367525
BD Vacutainer 5.0 ml, Re
Sarstedt Monovette, 8.5 ml
PS Tube Sarstedt 5ml, Ref : 55.476
Sarstedt Monovette 4.5 ml
Sarstedt Monovette 7.5 ml
Sarstedt Monovette 9.0 ml

Important indications

1. Minimum volume of samples in primary tubes

The procedure of the **InviMag® Virus RNA Mini Kit/ IG** is optimized for the isolation of viral RNA from up to 200 µl cell-free body fluids (serum, plasma) and rinse liquid from swabs as well supernatant from stool suspensions. It is advised to provide at least 550 µl sample tube to prevent pipetting distribution errors during processing.

2. Sample volume smaller than 200 µl

For samples of a smaller volume than 200 µl please fill the sample tube with PBS to a volume of minimal 400 µl.

3. Elution volume

The final processed sample volume is 200 µl which is eluted in 100µl Elution Buffer R (contains no EDTA).

Prevention of cross-contamination

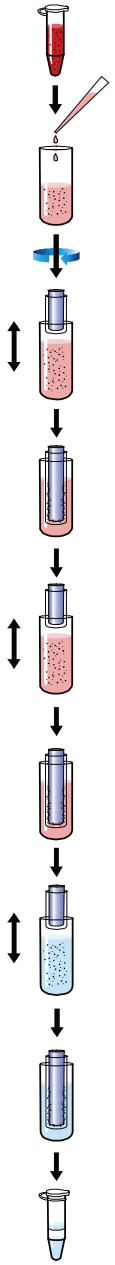
To comply with the demanding guidelines of *in-vitro* diagnostics we programmed the InviGenius® to route the pipettor in such a way that possible contamination-risks are minimized. However we recommend to apply the supplied well-strips and –foils beforehand (and afterwards on the used wells) on the unused wells of the Incubation Plate A and the Working-Plate B.

To prevent any form of salt-crystallization of used Lysis Buffer RV or Wash Buffer R1 wells it is recommended to reseal used wells after a run.

Scheme of the InviMag® Virus RNA Mini Kit/ IG

Add the primary tubes in the sample loading rack.

Add the Buffers in the Buffer loading rack.



200 µl sample is mixed with 400 µl **Lysis Buffer RV;** 40 µl **Proteinase K,** 40 µl **Carrier RNA** and 40 µl **MAP Solution B**. Incubation at elevated temperature is performed for 10 min.

450 µl **Binding Solution** is added to the lysate

RNA binds to magnetic particles

Magnetic separation

Washing of the particle fixed viral RNA with:
1 x 600 µl **Wash Buffer R1**
2 x 600 µl **Wash Buffer R2**

Magnetic separation

Elution of viral RNA in 100 µ **Elution Buffer R**

Magnetic separation and removal of **MAP Solution B**

Pure viral RNA

Preparing the samples for processing on the InviGenius®-system

Please read the instructions carefully and conduct the prepared procedure.

Important Note: *The protocol is optimized for the isolation of viral RNA from up to 200 µl of cell-free fluid. To prevent possible distribution errors we highly recommend using at least 400 µl of sample in total to ensure stable processing.*

1. Extraction of viral RNA from serum, plasma, cell-free body fluids
This type of sample can be processed directly without any preparations. Please make sure to supply at least 400 µl or dilute with PBS up to this volume.

RVIR_E100S200 (processed sample volume is 200 µl, elution volume 100 µl)

2. Extraction of viral RNA from swab samples
Rinse each swab with 500 µl cooled water or cooled PBS and load it into the InviGenius®.

RVIR_E100S200 (processed sample volume is 200 µl, elution volume 100 µl)

3. Extraction of viral RNA from supernatant of stool suspension
Pipet 600 µl ddH_2O in a 1.5 ml reaction tube (not provided).

Add a glass stick to the stool sample and transfer the adherent sample (size of a lentil) in the prefilled 1.5 ml reaction tube.

Resuspend the sample in the prefilled water.

Close the tube and vortex each sample vigorously until it becomes a homogenic suspension.

Centrifuge the samples for 5 min at 12.000 x g (13.400 rpm). Dip carefully the pipette tip about 0.5 mm below the surface and take from there 500 µl supernatant (prevent the aspiration of swimming particles) and transfer the sample in the sample tube and load it into the InviGenius® .

RVIR_E100S200 (processed sample volume is 200 µl, elution volume 100 µl)

Prevention of cross-contamination

To comply with the demanding guidelines of *in-vitro d*iagnostics we programmed the InviGenius® to route the pipettor in such a way that possible contamination-risks are minimized. However we recommend to apply the supplied well-strips and –foils beforehand (and afterwards on the used wells) on the unused wells of the Incubation Plate A and the Working-Plate B.

Preparing of the internal control for the InviGenius®-system

Please read the instructions carefully and conduct the prepared procedure.

Using an internal control (IC)

Using the InviGenius® Pathogen Extraction Kits in combination with commercially available amplification systems may require introducing an internal control (IC) into the purification procedure to monitor the efficiency of sample preparation.

Internal control DNA or RNA (IC) must be combined with Carrier RNA stock solution (or with Carrier RNA - Proteinase K - stock solution / InviMag® Universal Kit/ IG) in one mixture. For each sample the machine transfers a volume of 40 µl of the stock solution to the lysis mix.

The vials with PKC (Proteinase K and Carrier-RNA mixture) are dissolved with 800 µl RNase free water. Therefore an internal control for 20 samples should be added and added volume must be subtracted from the total volume

Example – Calculation:

Per Extraction 4.5 µl of a control is required

4.5 µl / RXN X 20 RXN = 90 µl

→ PKC stock solution has to be made by adding 800 µl – 90 µl = 710 µl RNase free water. Then 90 µl control DNA is added followed mixing.

Notes:

If the indication of amount per reaction is known, please calculate by using eluate and template volume.

If the internal control (IC) is stable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs (e.g., armored RNA), it can alternatively be added to the sample shortly before beginning sample preparation. But consider that a bigger amount of internal control is necessary when using bigger volumes of primary sample tubes.

If the internal control (IC) is naked DNA or RNA, it is unstable in plasma, serum, CSF, urine, respiratory samples, whole blood, stool, transport media, or on dried swabs and must not be added directly to the samples.

Refer to the manufacturer's instructions to determine the optimal amount of internal control (IC) for specific downstream applications. Using an amount other than that recommended may lead to wrong quantification results.

General overview of the InviGenius® System

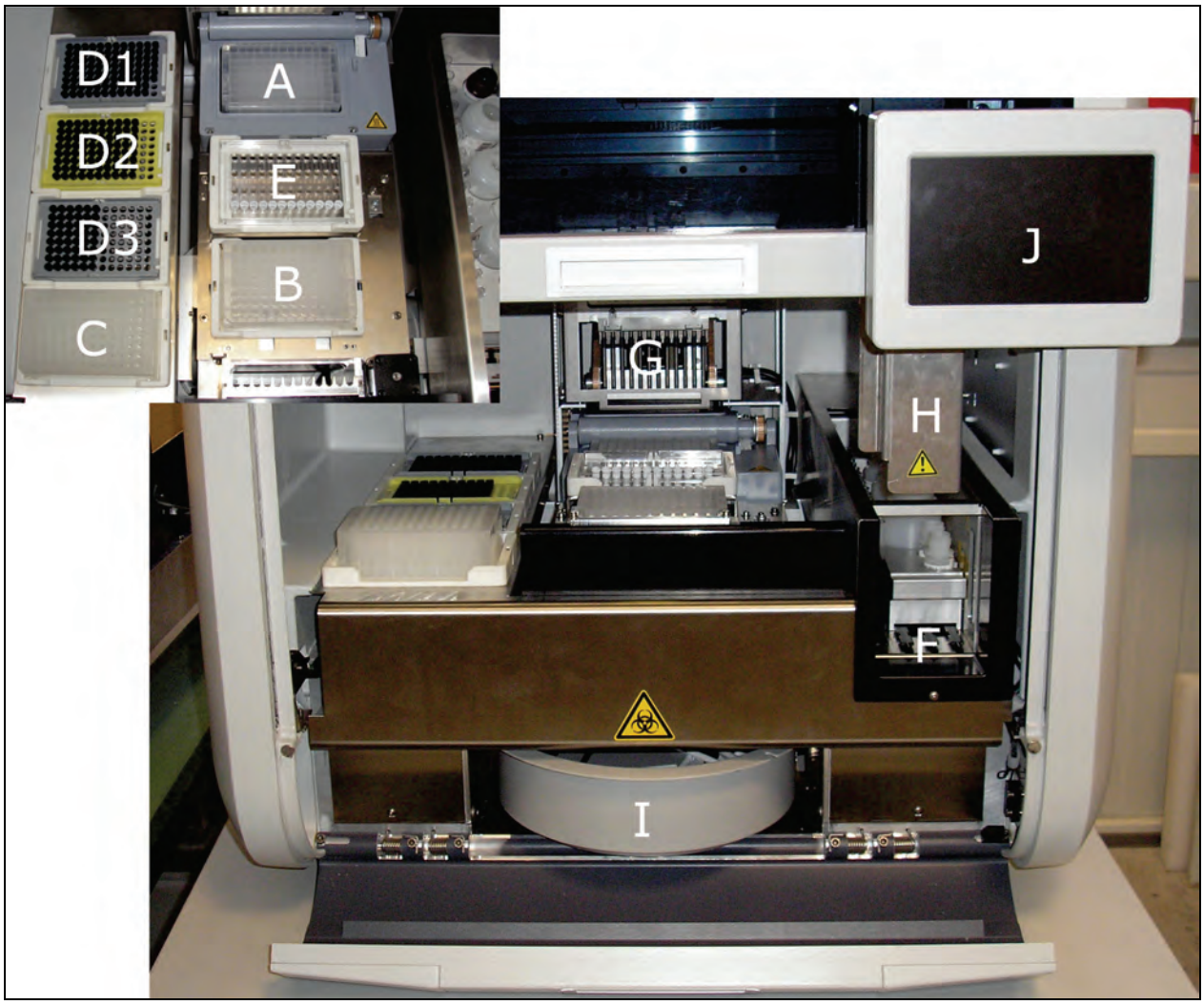


Figure 1: Frontal view of the InviGenius® System

There are three plate positions available in the InviGenius® system which can be loaded with corresponding plates: the incubator k (A), the working (B), and the eluate position (C).

Lysis is performed at the incubator position (A), whereas the washing and elution process is performed at the working position (B). The eluate - containing the extracted nucleic acids – will be finally transferred to the eluate position.

Additionally, there are three loading positions available for disposable tip trays (D1-D3) and one position (E) for the disposable sheaths. The loading bay (F) is located at the very right side of the instrument. The sample rack is loaded into the far left lane whereas the reagent rack is loaded into the right lanes of the loading bay.

The Magnetic Separation Head (MSH) (G) is located on top of the incubator lid (parking position). The fully automatic pipettor (H) is installed above the loading bay (parking position). The disposable waste tray (I) is located behind the lower cover of the InviGenius®.

Interaction with the InviGenius® instrument is performed by use of the touch LCD (J) located at the top front right side.

Preparing and loading of the InviGenius®-system

Preparing the reagents

Before you start, dissolve one vial of Proteinase K and Carrier RNA with 800 µl DNAse-free water, respectively.

Preparing the system

Turn on the InviGenius® system using the power switch located on the right back side of the instrument. The InviGenius® software can be started by double clicking the InviGenius® icon located on the desktop. Keep the door of the InviGenius® system closed during initialization.

After initialization of the InviGenius® system a login screen appears (Figure 1).

Log-in with the provided user name and password.

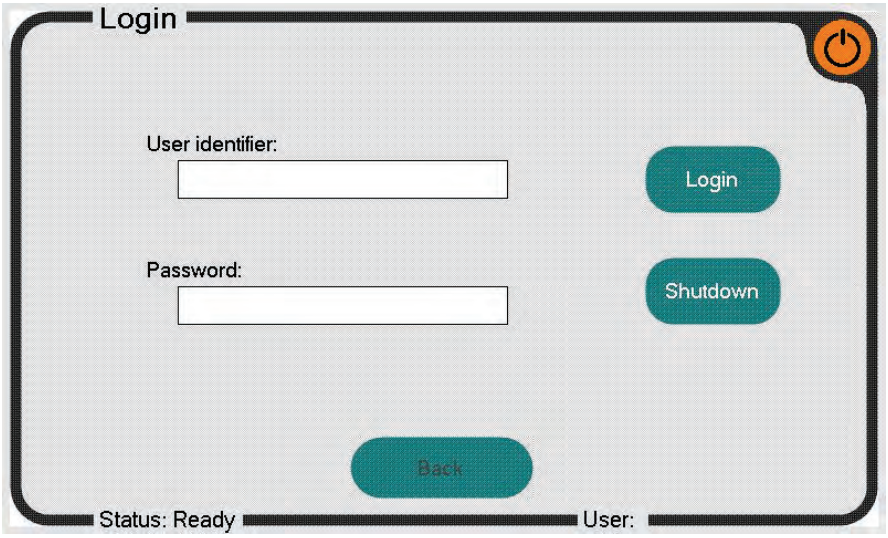


Figure 2: Login screen of the InviGenius® software

After login the main screen of the InviGenius® software is shown (Figure 2). Select “Loading” to start with loading of the system. Select “Processing” to define and run an assay if the system has already been loaded.

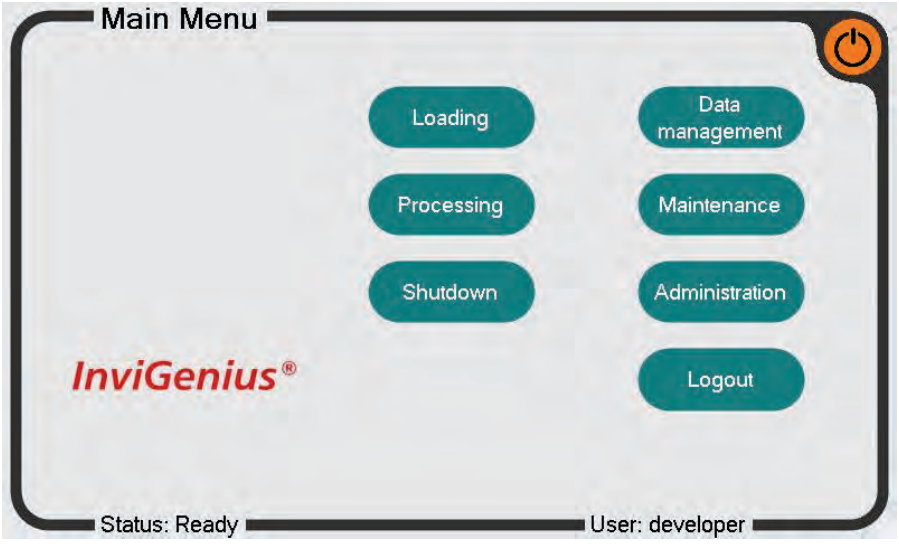


Figure 3: Main menu of the InviGenius® software

After selecting “Loading” the sample loading screen appears.

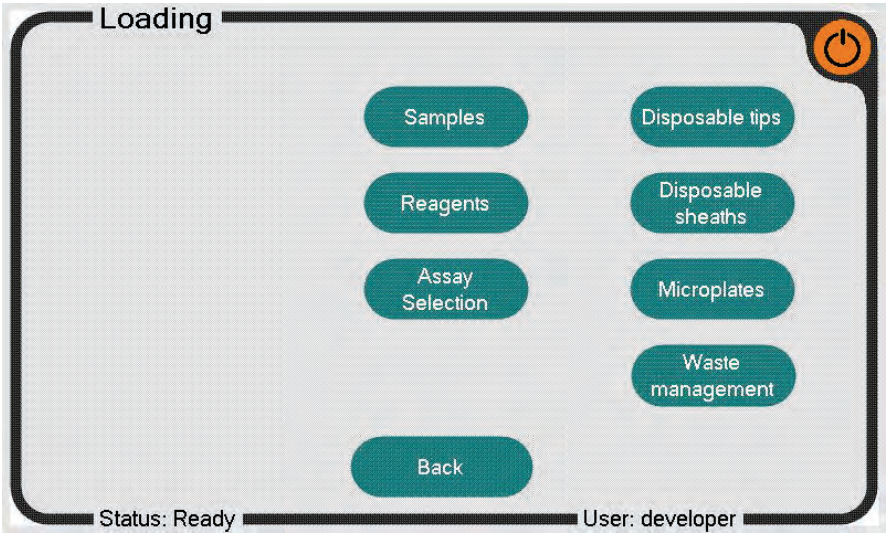


Figure 4: Loading screen of the InviGenius® software

Select “Samples” to proceed with the sample-loading-screen.

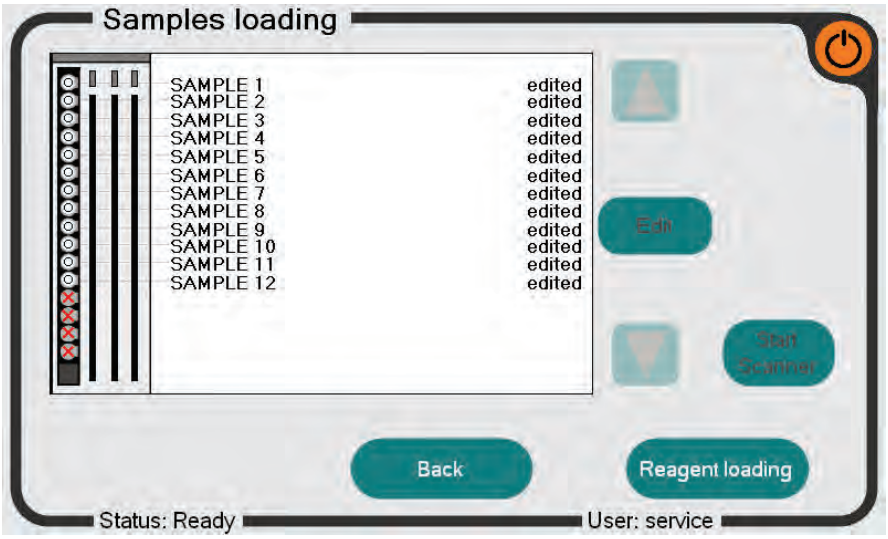


Figure 5: “Sample-loading” screen of the InviGenius® software

Please add the samples to the rack. Please decap the tubes before transfer to the loading rack.

If available the primary tubes should be used directly as sample tubes. If the samples are not provided in primary tubes, please prepare the sample rack with primary tubes that are prefilled with samples from which the viral DNA shall be extracted. Sample tubes are not provided with the kit and can be ordered at e.g. Sarstedt (order no. 55.476, 5 ml tubes, 75x12 mm, PS) or see recommendation at page 10, chapter: reagents and equipment to be supplied by user.

For each reaction a sample volume of 200 µl is processed. However, it is recommended that the total sample volume filled in the sample tubes should be at least 400 µl to ensure stable processing. Please take care, that only the first 12 positions of the sample rack can be processed due to the limited number of wells per row of the plastics. For correct identification of the sample tubes the unique bar codes must face to the bar code scanner located at the right side of the loading bay.

After inserting the sample rack in the very left lane of the loading bay, an updated screen will show the identifiers read from the sample bar codes (Figure 7). In case of unsuccessful sample identification, remove the rack, check the bar code orientation, and reinsert the rack

slowly. It is also possible to rename the samples by selecting the corresponding sample by using the arrow fields, followed by the “Edit” button.

After a certain time (about 5 min) the bar code scanner is inactivated. In that case the user has to restart the scanner with the “START SCANNER” button if the loading procedure is not finished.

After successful loading of the samples proceed with reagent loading by selecting “Reagent loading” on the bottom right hand side of this screen.

Reagent Loading:

The reagent loading process is analogous to the sample loading procedure.

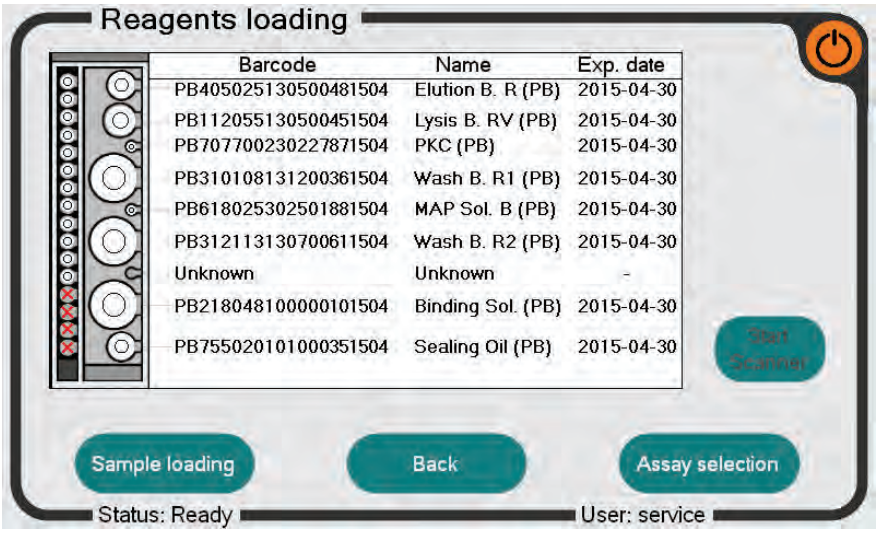


Figure 6: “Reagent-loading” screen of the InviGenius® software

Insert all provided reagents into the provided reagent rack of the InviGenius® system. Take care that the bar code labels face to the right side of the loading bay and decap the bottles and tubes. The order of the inserted reagents is not crucial because the type and position of a reagent is identified by the unique bar code. However, the possible loading positions are limited by the size of the used bottles.

After rack insertion the loading status of the reagents will be shown. In case of unsuccessful reagent allocation, remove the rack, check the bar code orientation and repeat the procedure slowly.

Assay Selection:

Select RVIR_E100_S200 assay and proceed with disposable tip loading.

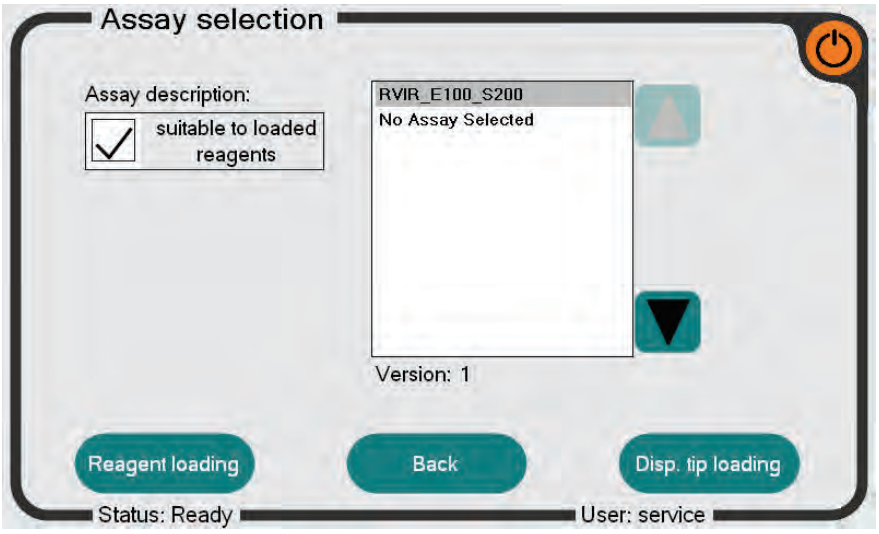


Figure 7: "Assay-selection" screen of the InviGenius® software

Disposable Tip Loading:

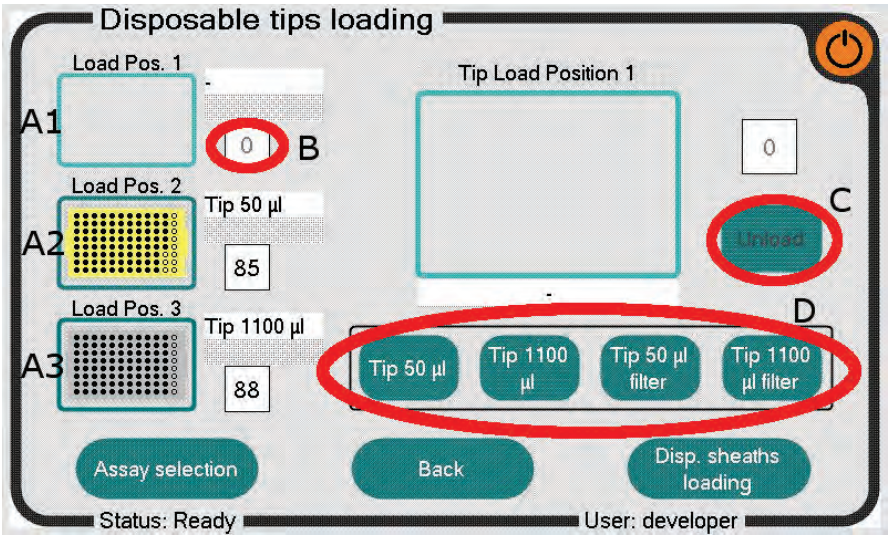


Figure 8: Disposable tip loading screen

There are three tip rack positions on the InviGenius® system (Fig. 10, A1-A3; corresponding to Fig. 3, D1-D3). Remaining tip-numbers are shown in B. Tip-numbers can be changed by pressing the number-field directly.

Empty tip-racks can be unloaded and reloaded by:

1.) Pressing the Loading-Position directly (The software will focus this loading position on the main screen)

2.) Pressing the Unload-Button C

3.) The loading-position can be refilled with a new tip-rack by pressing on the corresponding tip-rack on D

Each position can be filled either with 50 µl or 1100 µl filter or non-filter tips. However, for the Virus RNA assay, only 1100 µl filtered tips will be used.

Attention: ***It is very important to allocate the type of tips correctly in the software that have been loaded into the instrument. In case of false tip allocation, overfilling of the tip will irreparably destroy the pipettor head!***

All protocols should be used in combination with filter tips to ensure efficient prevention of sample or reagent cross-contaminations. STRATEC Molecular will give no guarantee or responsibility if contaminations occur due to the use of non-filtered tips.

Note: ***Disposable tips are not supplied within the kit. We recommend the use of validated conductive tips, which can be ordered at STRATEC Molecular. STRATEC Molecular offers 50 µl conductive tips (10x 96 pieces, order no. 5011120100) and 1100 µl conductive tips (10x 96 pieces, order no. 5011120200). Be sure that conductive tips are used otherwise the tip detection unit, installed in the pipetting unit, will reject the tips and no run will be possible.***

Disposable Sheaths Loading:

The sheaths are used as protection devices for the magnetic rods.

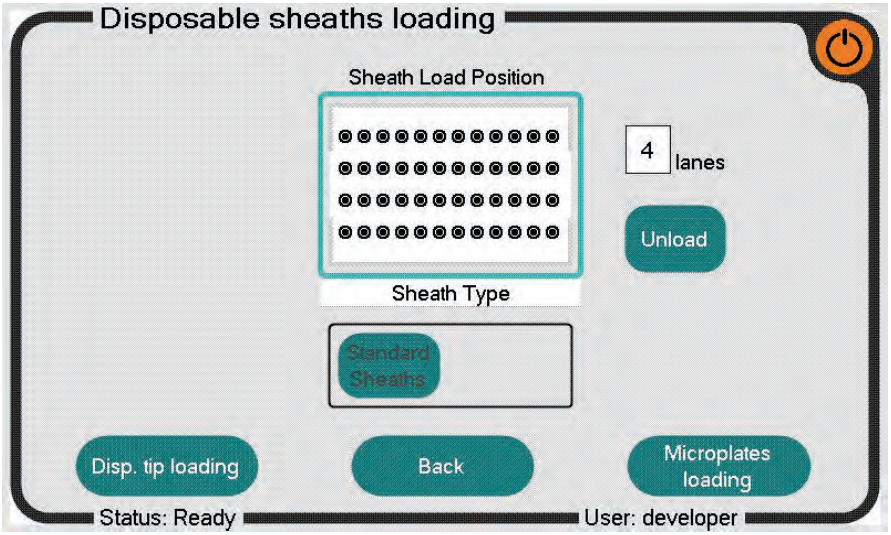


Figure 9: Disposable sheaths loading screen

The loading procedure of the disposable sheaths works analogous to the disposable tip loading screen. For a run, always 12 disposable sheaths (one row in the sheaths rack) are used, regardless of the processed sample numbers. This is done, to assure that the rods are always protected against contaminations.

In general, the number of sheaths supplied within the kit is sufficient for the amount of runs printed on the kit package. If you are lacking sheaths, they can be ordered separately at STRATEC Molecular (100 pieces bulk, order no. 5011120300 or 10 x 48 pieces, order no. 5011120400).

Comparable to the disposable tips loading it is possible to define the number of rows left in the tip rack by pressing on the displayed number area. Make sure that the disposable sheaths are loaded (and displayed) consistent to the manually loaded sheaths in the rack to ensure correct sheaths pick up. Don't remove single disposable sheaths within a row of the sheaths rack if less than 12 samples are processed within one run because there is a sheaths detection sensor installed in the device. If less than 12 sheaths picked up by the instrument a warning will be displayed and all picked up sheath will be discarded into the waste before a next row of sheaths will be picked up for testing.

To avoid contaminations, we strongly recommend to not wash/reuse any disposed sheaths!

Plate Loading:

Analogous to the previous loading screens, the Incubation Plate A, Working Plate A and Elution Plate E are loaded within the plate loading screen (Figure 9).

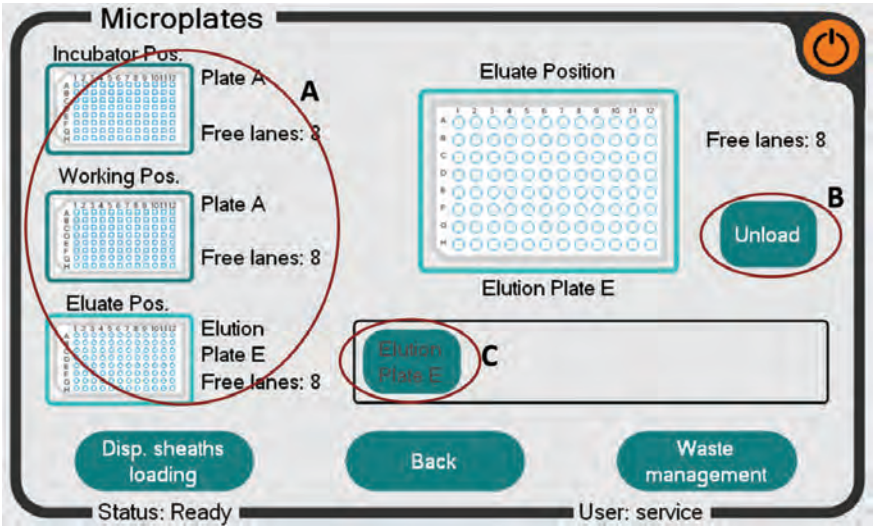


Figure 10: Plate loading screen

In general, the Incubation Plate A and Working Plate A (identical) are used at the incubator and working position whereas at the eluate position the Elution Plate E is used.

Used plates can be unloaded and reloaded by:

1.) Pressing the plate position directly (A). The software will focus at the plate position on the main screen.

2.) Pressing the “Unload” button (B)

3.) The plate can be reloaded by pressing on the offered plate in (C).

For a successful run the InviGenius® needs one free lane in the incubator position, seven free lanes in the working position and one free lane in the eluate position.

Please make sure that the depicted lanes on the monitor are consistent with the real lanes in the corresponding positions.

To avoid contaminations, we strongly recommend to not wash/reuse disposed plates!

Waste management:

Please make sure that the waste tray is capacity is sufficient for your planned assay. If not, empty the solid waste.

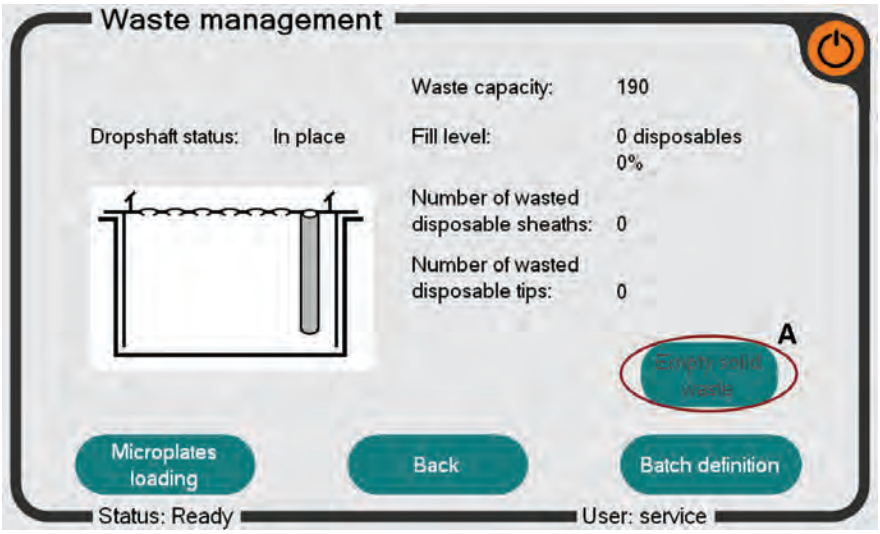


Figure 11: Waste-management-screen

If you have cleaned the waste tray, please use the “Empty solid waste” button (A).

Batch definition:

Please select the appropriate assay and check the samples you want to process in this run.

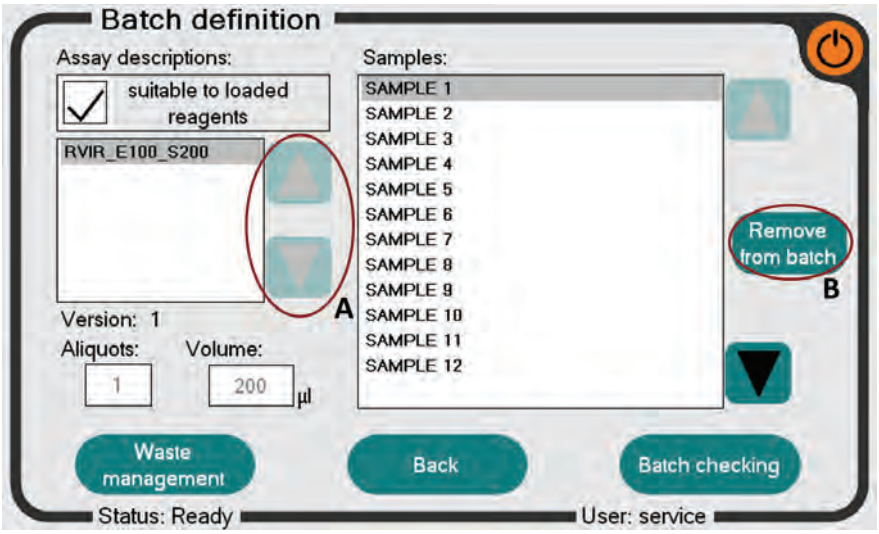


Figure 12: Batch-definition-screen

Please select the desired assay and recheck the allocated samples that should be processed in this run. It is possible to switch between the offered assays by using the two arrow buttons (A).

By default, all loaded samples are selected to be processed in this run. If samples have to be excluded from the batch, exclude them by selecting the corresponding sample and clicking on the “Remove from batch” button (B).

Batch checking:

This screen shows a summary of all checked disposables, samples and reagents in one informational screen. Please make sure that all required components are loaded correctly. In case of any error, the problem will be highlighted in by a red font. If no errors during the loading steps occurred, proceed by pressing the button “Batch processing”.

To solve any error, click on the red highlighted field and follow the instructions printed on the instrument screen.

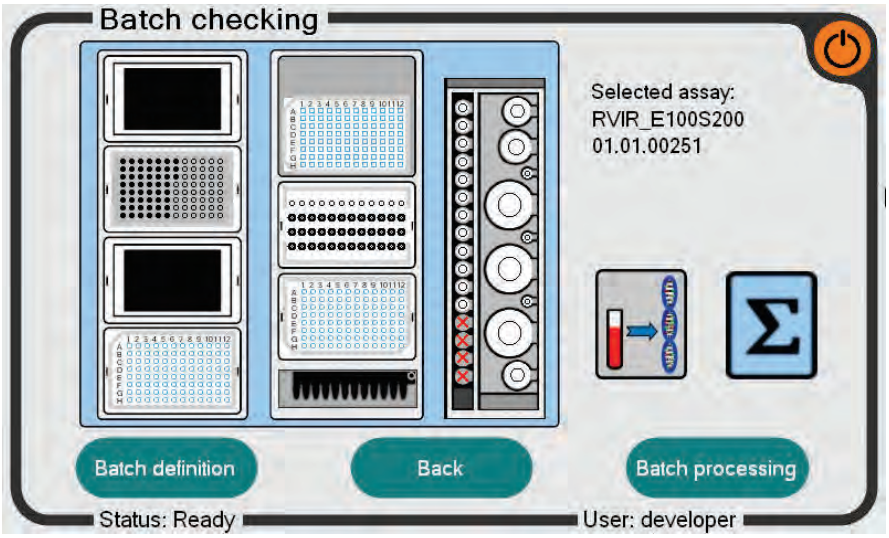


Figure 13: Batch-definition-screen

Batch processing:

After closing the system-door, the assay can be started by pressing the “Start”-Button (A). The door will be locked during the run and the system will start with sample processing. The door will only be unlocked after a run has been successfully finished or if an error occurs that requires user interaction. Do not try to force open the door during a run. This will cause an abort of the run!

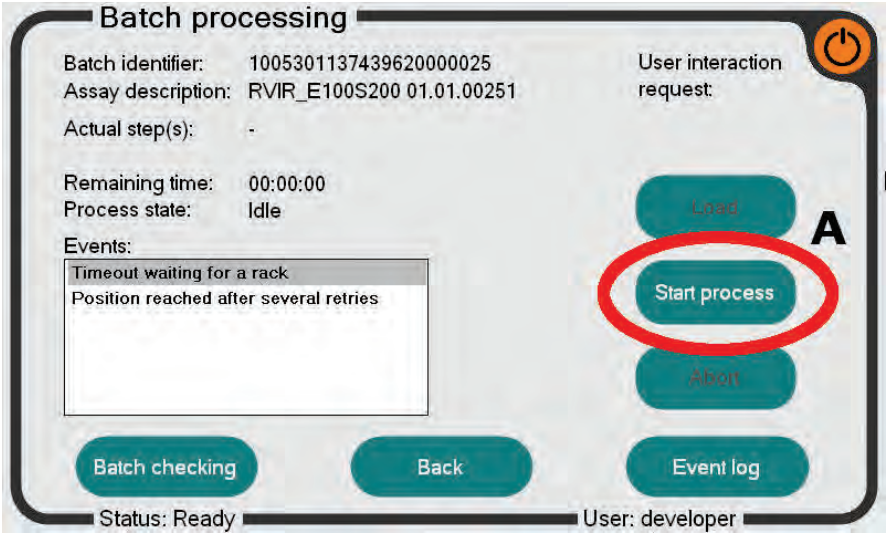


Figure 14: Batch-processing-screen

At the end of the run, the viral RNA-containing eluate is located in the appropriate eluate position and can be used for further applications.

After a run

After a run is completed and no additional run shall be started, unload all plates and reagents and store them according to GLP guidelines. Please keep in mind, that the plates could contain infectious material.

As with all medical/clinical and diagnostically equipment, all waste (liquids, tips, sheaths and plates) should be treated as potentially dangerous bio-hazard waste.

Daily maintenance (UV decontamination)

The InviGenius® system is equipped with an internal UV lamp (254 nm wavelength) that should be used daily either at the end of the working day or in the morning before a run is started. The suggested decontamination time is about 20 min. To start the UV decontamination go to the main menu of the InviGenius software and select “Maintenance”.

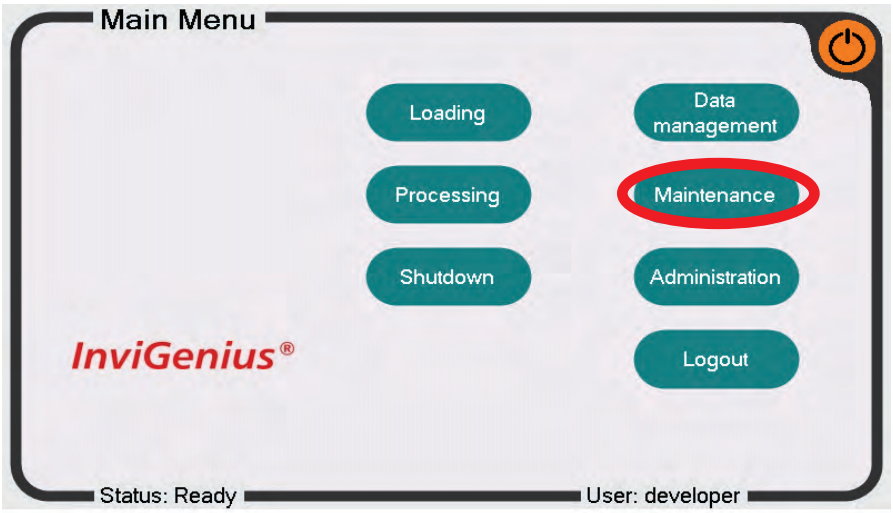


Figure 15: Main screen of the InviGenius® software

When the sub item “Maintenance” is opened, select “UV decontamination”

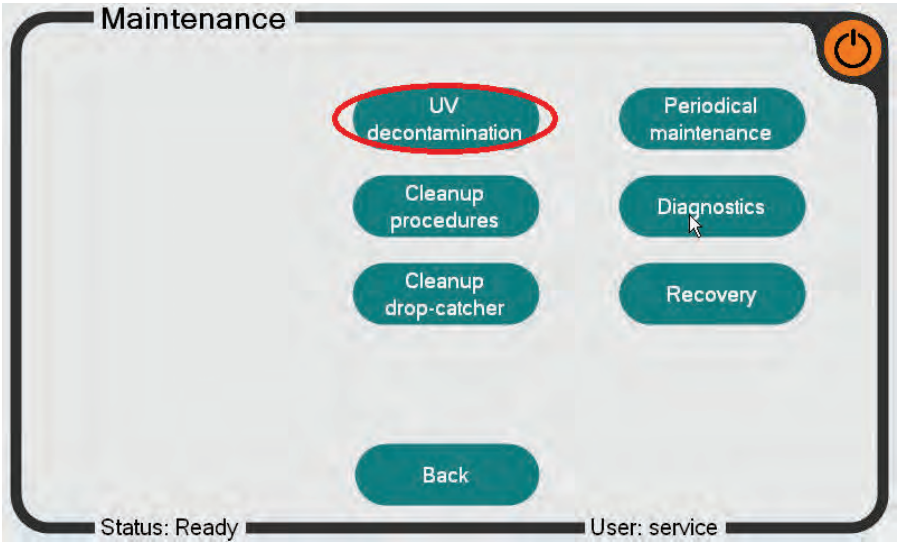


Figure 16: Maintenance screen of the InviGenius® software

In the UV decontamination menu adjust the exposure time (A) and finally press the “Start” button (B). During the decontamination process the instrument door will be locked to prevent any UV radiation release in the lab.

Warning: ***UV radiation is harmful. It causes serious burns of the skin and leads to irreparable damage of the eyes and skin. Ensure that no lab personnel is submitted to direct UV light. Do not try to force open the instrument door during the decontamination process.***

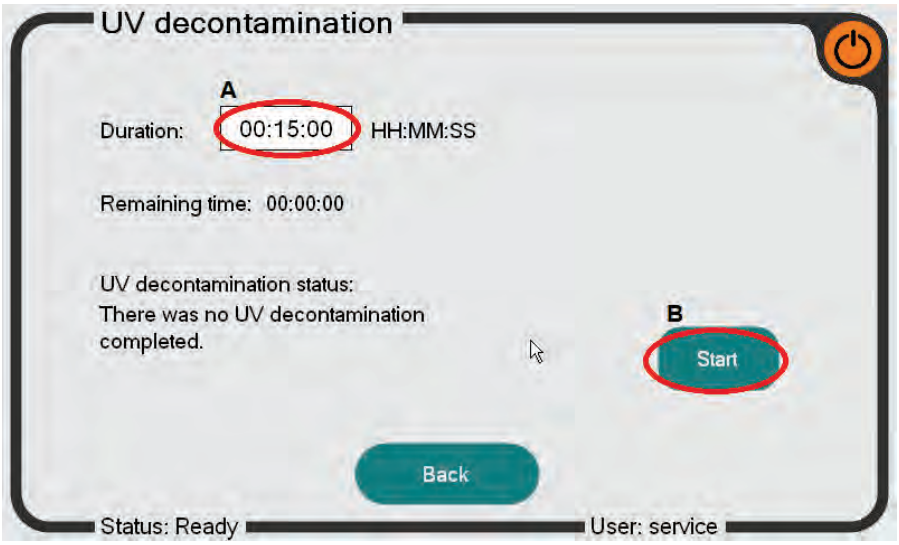


Figure 17: UV decontamination screen

When the decontamination is finished, go back to the main menu by using the “Back” button. The device is now decontaminated and can be either switched off or used for sample processing. We recommend decontaminating the instrument daily.

Appendix

Example data

To show the reproducibility of the viral RNA extraction using the InviGenius® system, three extractions were performed of an artificial Influenza-mastermix with different elution volumes (Runfiles: RNAVIR_100; RNAVIR_150 and RNAVIR_200; dead volume: 50µl). Real-time PCR was performed to quantify the extracted viral RNA.

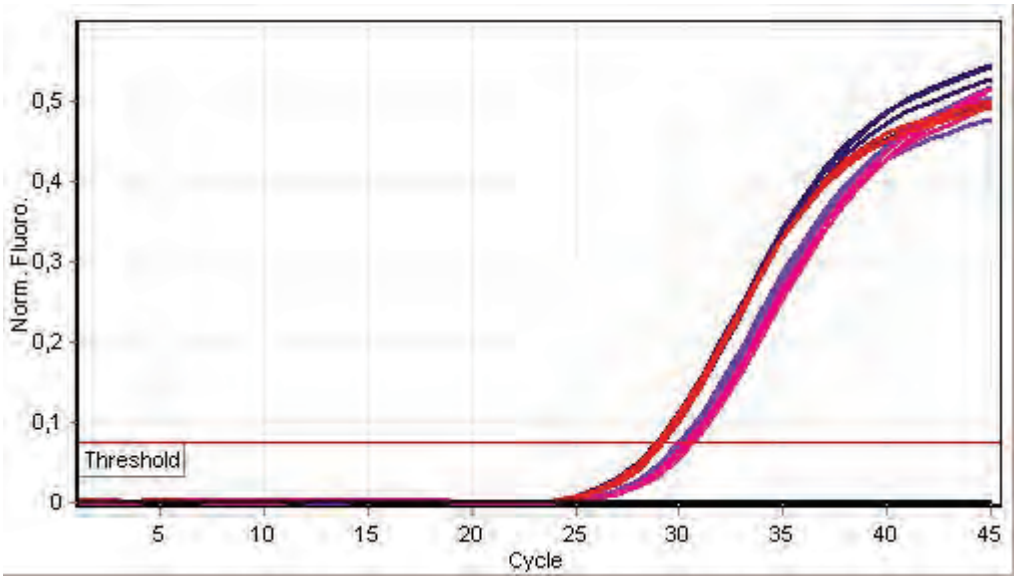


Figure 18: Real-time PCR-data of the 3 different runs.

No.	Name	Type	Ct	Rep. Ct	Rep. Ct Std. Dev.	Rep. Ct (95% CI)
1	RNAVIR_050	Unknown	29,1	29,05	0,16	[28,79 , 29,31]
2	RNAVIR_050	Unknown	28,9			
3	RNAVIR_050	Unknown	29,26			
4	RNAVIR_050	Unknown	28,94			
5	RNAVIR_100	Unknown	30,13	30,18	0,13	[29,97 , 30,39]
6	RNAVIR_100	Unknown	30,34			
7	RNAVIR_100	Unknown	30,21			
8	RNAVIR_100	Unknown	30,04			
9	RNAVIR_150	Unknown	30,52	30,6	0,19	[30,31 , 30,90]
10	RNAVIR_150	Unknown	30,72			
11	RNAVIR_150	Unknown	30,38			
12	RNAVIR_150	Unknown	30,8			
25	PTC	Positive Control	29,01	29,14	0,19	
26	PTC	Positive Control	29,28			
27	NTC	NTC				
28	NTC	NTC				

Table 1: Ct of three different diluted volumes protocols

The InviGenius® produced successful and reproducible results in three different runs with different elution volumes (50 µl remains in the plate).

General notes on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases has to be reduced as much as possible. Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification is to be carried out.

- Non-disposable plastics should be treated before use to ensure that it is RNase free. Plastic ware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase free water. You can also take chloroform-resistant plastic ware rinsed with chloroform to inactivate RNases.
- All buffers must be prepared from DEPC-treated RNase free ddH2O.
- When working with chemicals, always wear a suitable lab coat, disposable gloves and protective goggles.
- Change gloves frequently and keep tubes closed.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase free).
- Keep isolated RNA on ice.
- Do not use kit components from other kits with the kit you are currently using, unless the lot numbers are identical.
- To minimize the risk of infections from potentially infectious material, we recommend working under laminar air-flow for any steps before starting the extraction on the machine.

This kit should only be used by personnel trained in *in-vitro* diagnostic laboratory practice.

Storage of RNA

Purified RNA can be stored -80°C and is stable for years, e.g. precipitated and stored in 70% ethanol.

Troubleshooting

Problem	Probable cause	Comments and suggestions
pipetting distribution errors	pipetting of **PKC** failed	ensure that the lyophilized **PKC** is lyophilized with the appropriate volume of water before usage
	samples transfer failed / incomplete	the sample tube must contain at least 400 µl sample
	reagent / buffer transfer failed / incomplete	ensure that the supplied **Wash Buffers / Binding Solution** is filled up properly with either ethanol or isopropanol do not reuse bottles more often than described in Tab.1 because they will be rejected by the system
low concentration of extracted RNA	blood components settled	in case of large sample volumes (>>1 ml) carefully premix the sample tube before inserting it into the sample rack
	no/ too much ethanol added to **Wash Buffers**	ensure that the **Wash Buffers** have been filled up properly with ethanol as indicated in Tab. 1
degraded RNA	incorrect storage of starting material	ensure that the storage of starting material is correct avoid multiple freezing and thawing cycles of the material
	old material	ensure that the starting material is fresh or stored at appropriate conditions (for long time storage at –20°C)! avoid multiple thawing and freezing cycles of the material old material may contain degraded DNA
no assay selectable	combination of reagents from different kits / missing required reagent	make sure that only and all reagents belonging to one kit type are used. a combination of reagents belonging to different kit types is not supported
eluted RNA is brownish colored	Residual magnetic particles are left in eluate	centrifuge the eluate plate at full speed for 1 min and transfer supernatant to a new plate / tube

Ordering information

Product	Package size	Catalogue No.
InviMag® Virus RNA Mini Kit/ IG	8 x 12 preparations	2443120100
Related products		
Invisorb® Spin Virus RNA Mini Kit	50 preparations	1040300200
Invisorb® Spin Virus RNA Mini Kit	250 preparations	1040300300
Invisorb® Virus RNA HTS 96 Kit/ X	4 x 96 preparations	7143310300
Invisorb® Virus RNA HTS 96 Kit/ X	24 x 96 preparations	7143310400
InviMag® Virus RNA Kit/ KF96	2 x 96 preparations	7443300100
InviMag® Virus RNA Kit/ KF96	5 x 96 preparations	7443300200
InviGenius® and consumables		
InviGenius®	1 unit	5011100000
Starting Box I/ IG Sheath Box Conductive filter tips, 1 ml; 2 x 2 rack/ pack (384 pieces) 5 Waste Trays 120 sample tubes	1 box	2400110100
Sheath Bundle	10 x 48 pieces	5011100300
Sheaths	1000 pieces	5011100200
Conductive filter tips, 1 ml	10 x 96 pieces	5011100400
Waste tray/ IG	25 pieces	5011100100
Conductive filter tips, 1 ml	10 x 96 pieces	5011120200
Waste tray/ IG (disposable)	25 pieces	5011120500

Possible suppliers for Isopropanol:

Carl Roth
2-Propanol
Rotipuran >99.7%, p.a., ACS, ISO
Order no. 6752

Applichem
2-Propanol für die Molekularbiologie
Order no. A3928

Sigma
2-Propanol
Order no. 59304-1L-F

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1G5J02/IG/05/2015